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Interaction of *Candida albicans* with Human Leukocytes and Serum[1]

ROBERT I. LEHRER AND MARTIN J. CLINE

Cancer Research Institute and the Department of Medicine, University of California Medical Center, San Francisco, California 94122

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A quantitative assay of candidacidal activity based on differential staining of nonviable *Candida albicans* by methylene blue was developed and applied to studies of leukocytes from normal individuals and patients with fungal and other infections. Serum factors were necessary for optimal phagocytosis of *C. albicans* but lacked direct candidacidal activity. Normal human neutrophils (38 studies) killed 29.0 ± 7.4% of ingested *C. albicans* in 1 hr. Eosinophils and monocytes killed a smaller percentage. Neutrophil candidacidal activity did not require protein or ribonucleic acid synthesis by the leukocyte but was inhibited by anaerobic conditions, potassium cyanide, and colchicine. Leukocytes of a patient with hereditary myeloperoxidase deficiency and of three children with chronic granulomatous disease phagocytized *C. albicans* normally, yet failed to kill them. Our data suggest that the neutrophil can play an important role in resistance to *Candida* infection and that the lysosomal enzyme myeloperoxidase and its oxidant substrate hydrogen peroxide are the major participants in neutrophil candidacidal activity.

Although certain disorders and therapeutic procedures appear to alter man's susceptibility to fungal infection, the factors involved in either normal or altered responses to fungal challenge are incompletely defined. *Candida albicans* is representative of the group of opportunistic pathogens which cause disease in the impaired host (3, 5, 13, 44). This study was undertaken to develop a quantitative method capable of measuring the candidacidal activity of human leukocytes and serum and to define the metabolic pathways of the leukocyte which participate in the candidacidal process. The new method and results of its application to studies of leukocyte function and simplified in vitro models of *Candida* infection are presented.

MATERIALS AND METHODS

C. albicans. A strain of *C. albicans* (UC 820) was obtained from the Department of Clinical Pathology, University of California. It showed features typical of *C. albicans* (2, 27), including chlamydospore formation, rapid filamentation in the presence of serum (38), characteristic growth pattern on EMB agar, fermentation of dextrose and maltose with acid and gas production, lack of lactose fermentation, and failure to produce gas in sucrose. Stock cultures were maintained on Sabouraud agar slants at room temperature and were transferred every 2 months.

Test organisms were inoculated into 50 ml of Sabouraud 2% dextrose broth (Difco) and cultured at 33 C. Under these conditions, the *Candida* cells grew only in yeast phase. Only cultures with fewer than 5% nonviable organisms, as determined by dye exclusion (*vide infra*), were employed in assays. This condition was fulfilled by most cultures 3 to 14 days old. *Candida* cells were quantitated in a hemocytometer and resuspended in Hanks balanced salt solution (BSS) for use in candidacidal assays.

Leukocyte preparation. Peripheral venous blood containing 5 units of heparin per ml (Lilly, benzyl alcohol preservative) was mixed with half its volume of 3% dextran in normal saline (39) and allowed to sediment at room temperature for 30 to 45 min. The leukocyte-rich supernatant fluid was removed, centrifuged at 150 × *g* for 10 min, and washed twice with BSS containing 10% fetal calf serum and 5 units of heparin per ml. Leukocyte and absolute polymorphonuclear (PMN) cell concentrations were determined by hemocytometer count. Just before being added to the assay, the leukocytes were centrifuged at 150 × *g* for 8 min and resuspended in BSS at a final concentration of 10^7 PMN/ml. Erythrocyte contamination of the preparations was variable, usually approximating one red cell per white cell. Platelet contamination also varied, but usually was less than one platelet per five leukocytes.

Serum. Fresh serum was obtained from several normal group AB donors, stored individually in 4-ml volumes on dry ice, and used within 1 month. In some

[1] This work was presented in part at the 1968 Meeting of the American Federation for Clinical Research, Atlantic City, N.J. (Clin. Res. 16:331, 1968).

experiments leukocytes were studied in the donor patient's serum.

Neutrophil candidacidal assay. Equal volumes (0.25 ml) of AB serum, leukocyte suspension, and BSS were added to sterile plastic tubes (12 by 75 mm). All tubes were prepared in duplicate; a third tube containing all components except leukocytes served as a control. Sterile technique was used throughout.

The tubes were incubated for 10 min at 37 C. A 0.25-ml volume of *C. albicans* at a concentration of 10^7 yeast cells per ml was then added, and the tubes were rotated (30 rev/min) at 37 C for 60 min. After 15 min, a drop was taken for direct examination and preparation of stained smears to confirm that all added organisms had been ingested. At 60 min, 0.25 ml of 2.5% sodium deoxycholate (*p*H 8.7) was added to each tube. At this concentration, deoxycholate causes immediate lysis of the blood cells without damage to the *Candida* cells. Methylene blue, 0.01% in distilled water, was then added to achieve a final volume of 4 to 5 ml and a final dye concentration of about 0.0075% or 2×10^{-4} M. The *Candida* cell suspensions were centrifuged at $1,100 \times g$ for 15 min at 4 C and resuspended in about 0.5 ml of the residual supernatant fluid. Thereafter, the tubes were kept in an ice-water bath until they could be examined microscopically. At least 300 *Candida* cells from each tube were examined to determine the percentage stained. To derive the candidacidal activity due to the action of phagocytes, the percentage of stained yeast cells in the control tubes, usually 0.5 to 2.5%, was subtracted from that in the experimental tubes. All tubes were counted in a "blind" manner to avoid subjective bias.

Viable *Candida* cells, which were unstained, clearly differed from the nonviable organisms, which took a uniform, intense blue cytoplasmic stain. If *Candida* cells were left at 0 C in the presence of methylene blue for more than 3 hr, some of the organisms acquired a diffuse, faintly blue tinge; therefore, observations were concluded within this time period.

Candidacidal activity was independent of the *Candida*-neutrophil ratio over a range of 0.5 to 1.5 *Candida* cells per neutrophil and of serum concentrations in the range of 6 to 50%. When cells of *C. albicans* cultured for 72 hr or more were used, no multiplication occurred during the 60-min incubation period of the assay, ensuring that the percentage killed was directly proportional to the absolute number killed. Initial experiments revealed that leukocytes killed by repeated freezing and thawing lacked candidacidal activity in this system.

Monocyte candidacidal assay. Monocytes were isolated from heparinized peripheral blood of normal donors by previously described methods (8) and suspended at 10^6 cells per ml in medium TC 199 containing 30% fetal calf serum. Each of a series of Leighton tubes, some containing cover slips, was inoculated with 1 ml of the mixture and incubated for 1 hr at 37 C to allow the monocytes to adhere firmly to glass. The incubation medium was then decanted and replaced twice with warm, fresh TC 199 containing fetal calf serum to remove the nonadherent cells, predominantly lymphocytes. Finally, the medium was again decanted and replaced with 1 ml of BSS containing 2×10^6 *Candida* cells and 25% normal AB serum. After a 30-min incubation period, the monocytes were washed twice with warm BSS and serum to remove the nonphagocytized yeast cells; they were then incubated for an additional 60 min in fresh BSS containing AB serum. In the tubes without cover slips, the incubation was terminated by the addition of 0.25 ml of 2.5% sodium deoxycholate (*p*H 8.7) to lyse the monocytes. Methylene blue was added, and the suspensions were handled and counted as in the neutrophil assay. For assessment of phagocytosis, cover slips from the other Leighton tubes were stained and examined.

RESULTS

Differential staining of viable and nonviable Candida. We established the ability of methylene blue to specifically stain nonviable *C. albicans* in several ways. Usually, fewer than 5% of the cells in the *Candida* broth cultures were stained by 2×10^{-4} M methylene blue. If the yeast cells were killed by boiling for 10 min, all were intensely stained. Staining occurred promptly and was independent of *p*H in the range of 5 to 8. A representative mixture of heat-killed and viable *Candida* cells after exposure to 2×10^{-4} M methylene blue is shown in Fig. 1.

The specificity of the methylene blue staining of phagocytized *C. albicans* cells was studied as follows. After an inoculum of *Candida* cells was ingested by normal leukocytes, the leukocytes were lysed with deoxycholate, and methylene blue was added. A drop of the lysed suspension was placed on a slide containing a thin strip of Sabouraud agar, sealed with a cover slip, and observed microscopically at intervals for 8 hr. During several such experiments, unstained *Candida* cells were seen to bud and form microcolonies, whereas the blue-stained *Candida* cells always failed to bud.

The possibility that methylene blue was itself responsible for killing the phagocytized *Candida* cells that were stainable was excluded by the following experiment. Ingested *Candida* cells were again recovered from normal leukocytes by deoxycholate lysis and divided into duplicate portions. One portion was diluted with Sabouraud broth, and pour plates were made in Sabouraud agar for colony counts. The second portion was diluted with 0.01% methylene blue, and the concentration of *Candida* cells was determined in a hemocytometer. A single free cell, or two or more attached cells, was also classified as a *Candida* "unit," and the concentration of these units was ascertained. When such a unit contained at least one unstained cell, it was also counted as a "viable unit." As a control, the same procedures were followed for *Candida* cells that had not been

exposed to leukocytes. The observed colony counts and the concentration of viable units were in close agreement (Table 1).

Direct effects of methylene blue and sodium deoxycholate on C. albicans. In another series of experiments, we studied the direct effects of methylene blue and sodium deoxycholate on the viability and growth of *C. albicans*.

The effect of methylene blue upon the replicative ability of yeast-phase *C. albicans* was determined by inoculating Sabouraud broth containing various concentrations of the dye with 10^5 cells of *C. albicans* per ml of broth. After the mixtures had been incubated for 15 hr at 33 C, the concentration of *Candida* cells (C_t) was determined by hemocytometer count, and the percentage of stained cells was determined. Replicative ability was expressed in terms of the number of generations (n) undergone by the original inoculum (C_o) and was calculated by the relationship: $n = \log_2 (C_t/C_o)$. Methylene blue had a concentration-dependent inhibitory effect on the rate of multiplication of *C. albicans* (Table 2). At a concentration of 2.1×10^{-4} M methylene blue, multiplication occurred at about half the maximal rate. This concentration failed to stain *C. albicans* cells during the 15-hr incubation period.

The effect of deoxycholate on the growth rate of *C. albicans* was investigated in a similar manner. At concentrations between 0.25 and 0.5%, deoxycholate caused increasing gel formation in the Sabouraud broth but had only a slight inhibitory effect on the rate of multiplication of *C. albicans*. No effect was noted with concentrations of 0.25% or below.

The effect of methylene blue on germinative

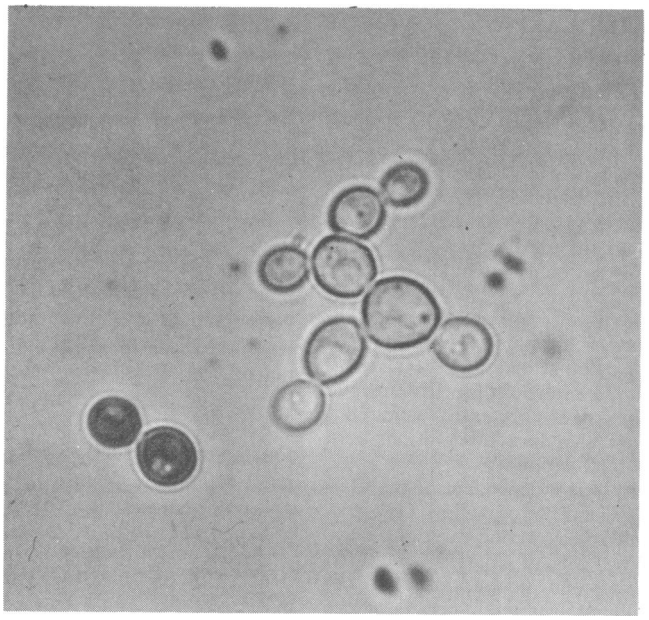

FIG. 1. *Mixture of heat-killed and viable. C. albicans after the addition of 2×10^{-4} M methylene blue. The dead organisms are uniformly stained.* ×600.

TABLE 1. *Concentration of viable Candida albicans as determined by colony count and by hemocytometer count after differential staining*

Condition	Colony count (colonies/ml)	Hemocytometer count[a]		
		Cells/ml	Units/ml	Viable units/ml
Phagocytosis	5.2×10^5	1.29×10^6	1.29×10^6	5.9×10^5
Control	1.41×10^6	1.81×10^6	1.48×10^6	1.39×10^6

[a] A single independent cell, or two or more attached cells, was also counted as a unit; a unit containing at least one cell that excluded 2×10^{-4} M methylene blue was also counted as a viable unit.

TABLE 2. *Effect of methylene blue on the growth of Candida albicans*[a]

Concn of methylene blue	*Candida* generations[b]	Germination	Stained
M	*No.*	%	%
0	8.4	95	0
5×10^{-5}	8.4	95	0
1×10^{-4}	6.7	95	0
2.1×10^{-4}	4.4	91	0
4.2×10^{-4}	2.9	41	4
8.4×10^{-4}	1.1	NT	38
1.7×10^{-3}	0.5	1	61
3.3×10^{-3}	0.3	NT	94

[a] NT = not tested.

[b] Calculated as $\log_2 (C_f/C_o)$, where C_f = final *Candida* cell concentration, and C_o = original *Candida* cell concentration.

ability was examined by inoculating *C. albicans* into Sabouraud broth containing 20% fetal calf serum and various concentrations of dye and incubating the tubes for 4 hr at 37 C. Germination was not significantly inhibited by 2.1×10^{-4} M methylene blue (Table 2).

In an additional experiment, Hanks BSS containing 2×10^{-4} M methylene blue and 0.125% sodium deoxycholate was inoculated with *C. albicans* and incubated for 4 hr at 0 or 37 C. No decreases in colony count were observed under these conditions.

It was concluded from these studies that the reagents, in the concentrations employed in the candidacidal assay, had no direct candidacidal activity.

Direct effect of human serum on C. albicans. In further experiments, 10^6 yeast-phase *C. albicans* cells were added to 1 ml of various normal sera and incubated at 37 C. A characteristic series of events occurred which were followed by direct microscopic observation and simultaneous colony counts. The viable *Candida* cells germinated after a few hours and then grew vigorously in a mycelial phase (Fig. 2). Initially, the colony counts remained constant and correlated well with direct hemocytometer counts of viable units. After a 3- or 4-hr incubation period, the organisms, now growing in mycelial phase, became aggregated into large, tangled masses containing hundreds of cells, and both the colony count and the number of viable units dropped sharply from initial levels (Fig. 2d). It was apparent that the fall in both colony count and concentration of viable units resulted from the agglutination of previously independent organisms into mycelial masses. During the time the colony count was falling, most *Candida* cells continued their pseudohyphal proliferation.

Effect of serum factors on phagocytosis. Serum factors were required for efficient phagocytosis of *C. albicans* by human neutrophils. Under standard assay conditions, all the added *Candida* cells were phagocytized within 10 min. Despite fluctuations in the relative proportions of eosinophils and monocytes in various preparations, over 90% of the yeast cells were consistently ingested by neutrophils. When the serum component of the incubation mixture was replaced by BSS or human serum albumin preparations, phagocytosis was virtually absent over a 60-min period. Comparison of the phagocytic process in the presence of 25% native serum or heat-inactivated (56 C, 30 min) normal human serum indicated that heat-labile factors accelerated the rate of phagocytosis of *Candida* cells by neutrophils (Fig. 3).

Effect of serum factors on killing of intracellular Candida. To test the possibility that serum factors might influence the fate of phagocytized *Candida* cells, it was necessary to devise conditions that would ensure comparable rates of phagocytosis when different serum preparations were used as opsonins. This was accomplished in the following manner. Cells of *C. albicans* were incubated for 30 min at 37 C in (i) native serum, (ii) heat-inactivated serum, (iii) serum containing 0.01 M disodium ethylenediaminetetraacetate (EDTA), or (iv) Hanks BSS. The *Candida* cells were then washed twice with cold BSS, resuspended in BSS at a concentration of 10^7/ml, and added to leukocytes suspended in serum-free Hanks solution to achieve a neutrophil-yeast cell ratio of 1:1. The tubes were centrifuged at $190 \times g$ for 5 min so that *Candida* cells and leukocytes were packed together in a small button, incubated for 15 min at 37 C, and then shaken to resuspend the cell button. Examination of stained smears made immediately after resuspension indicated that approximately 80 to 90% of the organisms in tubes i, ii, and iii were phagocytized, whereas phagocytosis in tube iv varied from 5 to 25%. The tubes were incubated for an additional 1 hr, and the percentage of nonviable *Candida* cells in each was determined. The ability of neutrophils to kill ingested cells of *C. albicans* did not depend on prior exposure of the organisms to heat-labile or EDTA-inhibitable serum factors (Table 3).

Metabolic inhibitors and other chemical agents. The assay system was used to determine the effect of anaerobiosis and of various agents on the candidacidal activity of normal neutrophils. Potassium cyanide, colchicine, puromycin, actinomycin D, and chloroquin were prepared in BSS and stored at −20 C until used. Hydrocortisone sodium succinate and tetracycline were incorporated into BSS just before each assay. In these

experiments, the incubation period before the addition of *C. albicans* was lengthened to 30 min.

Candidacidal activity was virtually absent under anaerobic conditions and was considerably diminished by 10^{-3} M cyanide or 10^{-4} M colchicine (Table 4). Preincubation with puromycin, actinomycin D, tetracycline, or hydrocortisone (1 μg/ml) failed to affect the candidacidal activity

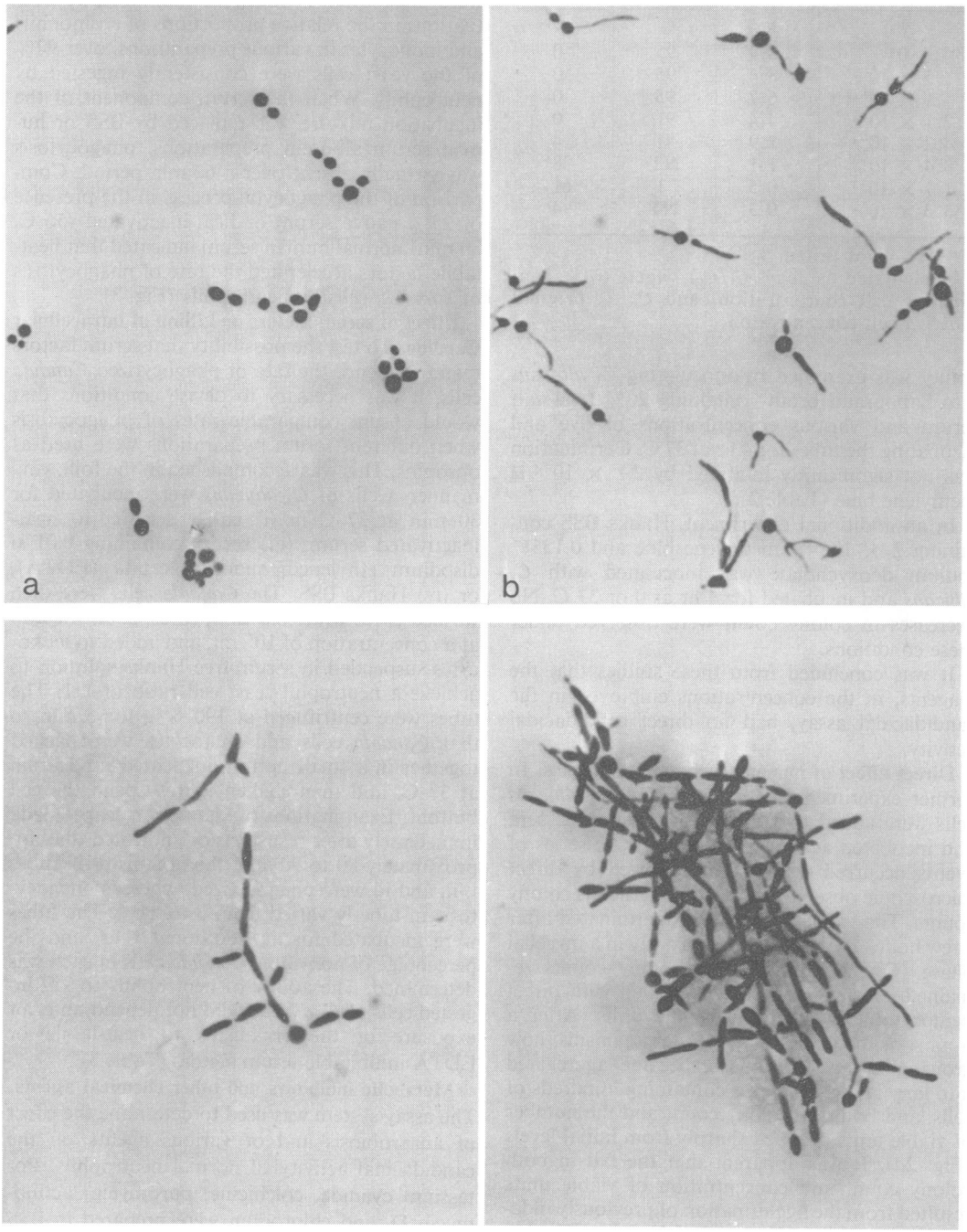

FIG. 2. *Effect of serum on the morphology of yeast-phase C. albicans. (a) Normal serum (1 ml) inoculated with 10^6 yeast-phase C. albicans. (b) After incubation at 37 C for 2 hr, showing formation of pseudo-germ tubes. (c) Continuation of growth in mycelial phase. (d) After incubation for 3 to 4 hr, showing aggregation of mycelial-phase organisms; simultaneously a fall in colony count occurred.* ×600.

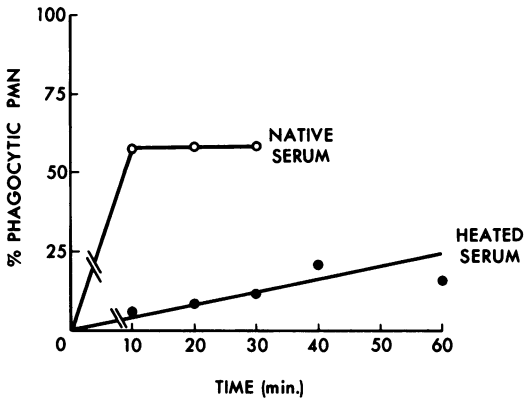

FIG. 3. *Accelerating effect of heat-labile serum factors on the phagocytosis of C. albicans by normal human neutrophils. In this study a yeast cell to neutrophil ratio of 1:1 was used. When all the added organisms had been ingested, the phagocytic neutrophils contained a mean of 1.7 Candida cells per leukocyte.*

TABLE 3. *Effect of serum factors on neutrophil candidacidal activity in two representative experiments*

Opsonizing agent	Percentage of *Candida* killed	
	Expt 1	Expt 2
Hanks BSS	8.6	2.7
Normal serum	34.4	22.3
Normal serum + EDTA	39.4	23.5
Heat-inactivated normal serum	40.1	24.3

of the neutrophils. When the cells were preincubated with hydrocortisone in a concentration of 30 μg/ml or with 10^{-6} M chloroquin, relatively slight decreases in candidacidal activity were observed. None of the agents tested impaired either phagocytic activity or leukocyte viability as measured by trypan blue exclusion.

Candidacidal activity of human leukocytes. The assay system was applied in studies of leukocytes from 48 normal subjects (hospital personnel and hematologically normal hospitalized patients lacking evidence of undue susceptibility to infection), 10 patients with various fungal diseases, and three patients with chronic granulomatous disease. The results are summarized in Fig. 4.

In assays with AB serum, the neutrophils of 38 of the normal subjects killed 29.0 ± 7.4% of the ingested *C. albicans* in 1 hr (median value, 29.1%; range, 18.5 to 46.4%). Neutrophils from the other 10 normal subjects, assayed in their own serum, killed 28.7 ± 4.6%. In assays on peripheral blood monocytes from six of the normal subjects, employing preparations of greater than 95% purity, only 4 ± 2% of the phagocytized *Candida* cells were killed. Eosinophilic leukocytes from a patient with a syndrome resembling eosinophilic leukemia (7) killed 12.4%.

Normal neutrophil candidacidal activity was found in 7 of the 10 patients with fungal diseases (Fig. 4). Of the three with impaired activity, one was a woman who was receiving steroid therapy

TABLE 4. *Effect of metabolic inhibitors and other chemical agents on the candidacidal activity of normal neutrophils*

Agent	Concn	No. of experiments	Candidacidal activity (% control)
Nitrogen atmosphere	100%	6	10 ± 3
Potassium cyanide	10^{-3} M	5	32 ± 11
Colchicine	10^{-4} M	6	57 ± 7
Puromycin	40 μg/ml	3	91 ± 5
Actinomycin D	0.5 μg/ml	4	105 ± 11
Tetracycline	50 μg/ml	3	103 ± 15
Hydrocortisone	1 μg/ml	5	99 ± 7
	30 μg/ml	5	88 ± 6
Chloroquin	10^{-6} M	5	85 ± 11

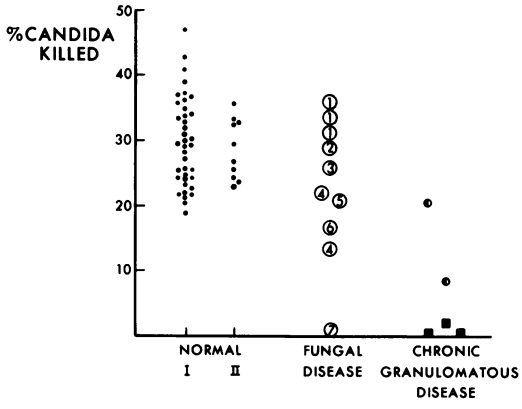

FIG. 4. *Percentage of C. albicans cells killed in 1 hr by normal neutrophils and neutrophils from patients with fungal diseases or chronic granulomatous disease. Normal I and II: neutrophils from 38 and 10 subjects, respectively. The patient's own serum was used for assays on normal II neutrophils; normal AB serum was used for assays on all other neutrophils, including normal I cells. Fungal disease (10 patients): 1 indicates disseminated coccidioidomycosis; 2, mucormycosis (and lymphosarcoma); 3, aspergilloma (and sarcoid); 4, cryptococcal meningitis; 5, Candida granuloma; 6, sporotrichosis (and lymphosarcoma); 7, systemic candidiasis. Chronic granulomatous disease (three patients): ■, affected male patient; ◑, clinically unaffected mother.*

for lupus nephritis and had cryptococcal meningitis following staphylococcal septicemia. The second patient had indolent sporotrichosis complicating long-standing lymphosarcoma with moderate neutropenia. The neutrophils of both patients phagocytized *C. albicans* normally but showed moderate depression of candidacidal activity. Neutrophils from the third patient, who had disseminated candidiasis, ingested cells of *C. albicans* normally but were unable to kill them. These leukocytes were found to be completely deficient in the enzyme myeloperoxidase (R. I. Lehrer and M. J. Cline, Clin. Res. **16**:331, 1968). Further details on this case will be published separately.

The neutrophils of three male children with chronic granulomatous disease, assayed in autologous or normal serum, phagocytized cells of *C. albicans* normally but failed to kill the ingested organisms. Myeloperoxidase was histochemically demonstrable in the leukocytes of all three. Deficient leukocyte candidacidal activity, intermediate between the normal and the chronic granulomatous disease level, was found in the mother of one patient (Fig. 4).

DISCUSSION

Certain limitations of colony-count methods encouraged us to evaluate alternative approaches for use in studies involving *C. albicans* and led to the methylene blue exclusion technique described in this report. A necessary assumption underlying colony-count methods is that a constant proportionality exists between the number of colonies formed and the number of viable organisms in the sample tested. For most purposes, the fact that colony-forming units rather than individual microbial cells are counted does not alter the validity of colony counting (21). Under some circumstances, however, the sensitivity or accuracy of the method is impaired. For example, in studies on the fate of *C. tropicalis* and *C. guilliermondi* phagocytized by human leukocytes, Louria and Brayton (22) detected no fall in colony count after a 4-hr incubation period and concluded that the ingested fungi survived within the leukocyte. *Candida* species, however, reproduce by budding, and cultures commonly include many short chains composed of two, three, or four attached viable yeast cells. If only a single cell in such a chain survived phagocytosis, it could give rise to a colony, thereby concealing the fact that other cells in the chain had been killed. With the methylene blue exclusion technique, this limitation in sensitivity is not a factor, since each cell of the chain is assessed individually. Differential staining procedures were first employed by Metchnikoff in 1887 to discriminate between viable and nonviable microorganisms (31) and have been applied by subsequent investigators to yeast (11, 14, 33) as well as to bacteria (21).

Louria and Brayton also described a substance in normal human serum or plasma that is lethal in vitro for *C. albicans*, but not for other *Candida* species (23). The assay for this substance is performed by inoculating 1 ml of serum with 10^6 *C. albicans* cells and incubating the mixture for 6 hr. When we performed this assay, with most normal sera tested we also noted the striking fall in colony count that Louria and Brayton attributed to the action of a specific lethal factor. Our data, however, suggest that the fall results from processes of mycelial growth and subsequent agglutination rather than from operation of a lethal factor. Several workers have shown that serum will induce yeast-phase *C. albicans* to form pseudo-germ tubes and grow in the mycelial phase (6, 26, 40). This change occurs in a few hours, is specific for *C. albicans*, and has been proposed as a rapid means of differentiating *C. albicans* from other *Candida* species (40). The species-specificity of the effect would also account for the apparent specificity of the "lethal" serum factor. Chilgren et al. (6) recently reported similar results and conclusions.

We found that, although serum lacked direct candidacidal activity, it contained heat-labile factors necessary for optimal phagocytosis by neutrophils. A similar effect of heat-labile opsonins on the phagocytosis of pneumococci by leukocytes was reported by Ward and Enders (43). The subject of heat-labile opsonins was reviewed by Hirsch and Strauss (15). Our observation that phagocytized *Candida* cells opsonized by normal serum, heat-inactivated serum, or normal serum containing EDTA were killed equivalently by normal neutrophils suggests that adsorbed complement or other heat-labile serum components fail to alter the intracellular fate of the ingested organism. Essentially similar findings were described by Craig and Suter (9) in their report on the staphylocidal ability of human neutrophils.

In our studies, considerable difference was found between the candidacidal abilities of normal human neutrophils and monocytes. Whereas neutrophils killed 29.0 $\pm$ 7.4% of the ingested *C. albicans* in 1 hr, monocytes killed only 4 $\pm$ 2%. Neutrophil candidacidal activity was not depressed by actinomycin D, puromycin, or tetracycline, and thus appears not to require ribonucleic acid or protein synthesis. Our observation (R. I. Lehrer and M. J. Cline, Clin. Res. **16**:331, 1968) that human neutrophils lacking MPO, a lysosomal enzyme (38), failed to kill ingested *Candida* cells suggests that MPO is an

essential participant in the neutrophil candidacidal process. The inhibiting effects of anaerobiosis, cyanide, and colchicine on the candidacidal activity of normal neutrophils may be related to their interaction with MPO.

After phagocytosis, normal neutrophils display a burst of oxygen consumption, insensitive to cyanide (37), which is apparently linked to hydrogen peroxide generation (18, 34). Although hydrogen peroxide is directly toxic to many microorganisms, its lethal effects may be greatly enhanced by MPO when suitable halides are present (19, 20, 30). Anaerobic conditions inhibit neutrophil candidacidal and bactericidal activity (30, 34), perhaps by curtailing the generation of hydrogen peroxide, the oxidant substrate of MPO. Cyanide ion is a direct inhibitor of the hemoprotein MPO (1). Colchicine, also effective as a candidacidal inhibitor, has a complex action on phagocytic neutrophils, leading to inhibition of oxygen consumption, degranulation, and hexose monophosphate shunt activity (12, 29). Colchicine also decreases the oxidation of sodium urate-6-^{14}C crystals phagocytized by human neutrophils (13), a metabolic step catalyzed by MPO (28). In addition, we found that highly purified human MPO forms a potent candidacidal system when combined with low concentrations of hydrogen peroxide and various halides (R. I. Lehrer and M. J. Cline, Clin. Res. **17**:121, 1969). Perhaps the limited ability of human monocytes to kill ingested *C. albicans* is a consequence of their relative paucity of MPO, clearly apparent in routine peroxidase stains of peripheral blood cells.

Although cellular processes apparently play a primary role in natural immunity against *Candida* infection, the relative contributions of phagocytic and immunogenic host cells have not been resolved. In candidiasis, as in other systemic mycoses, the presence of an augmented antibody response has been successfully used to diagnose disseminated disease (41). Brody and Finch (4) employed a sensitive immune adherence technique to study the distribution of antibody reacting with *C. albicans* in normal subjects and patients with neoplastic disorders. They found titers exceeding 1:4,000 in most sera tested, including the serum of a patient with acute leukemia and disseminated candidiasis. Dobias (10), in summarizing previous attempts to protect animals against *Candida* infection by active or passive immunization, noted that the results had been unsuccessful in general; however, he was able to induce partial protection in mice by immunizing them with large doses of *C. albicans* cell walls.

Hypogammaglobulinemia of the Bruton type and of the "acquired" type is associated with frequent bacterial infections but rarely with disseminated fungal infections (36). In one case, that of a child with the Swiss type of familial lymphocytopenic agammaglobulinemia, disseminated candidiasis followed antibiotic therapy for bacterial infection (42). Although the patient's immunological deficiency cannot be discounted as a predisposing factor, antibiotic therapy has been reported to precede disseminated candidiasis in children (17).

It recently became apparent that several disorders may be associated with defective neutrophil phagocytic or microbicidal function (16, 32, 35, 44; C. A. Alper et al., J. Clin. Invest. **47**:1a, 1968). In addition to our patient with hereditary MPO deficiency, our three patients with chronic granulomatous disease had adequate numbers of circulating neutrophils which displayed normal phagocytic ability yet failed to kill any of the ingested *C. albicans*. This candidacidal defect is similar to the well-documented inability of the neutrophils of chronic granulomatous disease patients to kill many species of bacteria (35). Although such neutrophils possess MPO, apparently they fail to generate hydrogen peroxide, its oxidant substrate, after phagocytosis (16).

Substantial clinical and experimental evidence indicates that the neutrophil is a major participant in the host response to systemic *Candida* infection. In experimental *Candida* infections, the disappearance of organisms from the tissue lesions parallels the appearance of neutrophils (24). Leukopenia is a common predisposing factor in disseminated candidiasis in man (3, 13, 25, 45). Finally, the present studies establish that normal neutrophils can kill ingested *C. albicans*, although serum itself lacks candidacidal activity.

The candidacidal assay described in this report provides a relatively simple and potentially useful means of screening for and studying leukocyte microbicidal deficiencies. Its use in the present studies has clarified some of the interactions among *C. albicans*, leukocytes, and serum that form an important component of host response to *Candida* infection.

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